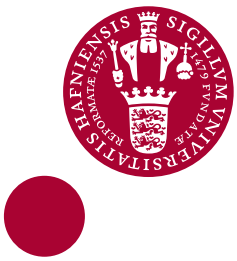

Generation of induced pluripotent stem cells, KCi001-A derived from a Bardet-Biedl syndrome patient compound heterozygous for the BBS1 variants c.1169T>G/c.1135G>C

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Lab resource: Stem Cell Line

Generation of induced pluripotent stem cells, KCi001-A derived from a Bardet-Biedl syndrome patient compound heterozygous for the *BBS1* variants *c.1169T > G/c.1135G > C*

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A B S T R A C T

Bardet-Biedl syndrome (BBS) is an autosomal recessive ciliopathy with a wide range of symptoms including obesity, retinal dystrophy, polycystic kidney disease, polydactyly, hypogonadism and learning difficulties. Here we describe the successful generation of an induced pluripotent stem cell (iPSC) KCi001-A from a BBS patient compound heterozygous for two disease causing *BBS1* variants c.1169T > G, p. (Met390Arg)/c.1135G > C, p.(Gly370Arg).

Resource table

Unique stem cell line identifier	KCi001-A
Alternative name(s) of stem cell line	BBS1 Clone10
Institution	Kennedy Center, Rigshospitalet
Contact information of distributor	Lisbeth Birk Møller, Lisbeth.Birk.Moeller@regionh.dk
Type of cell line	Induced pluripotent stem cell line (iPSC)
Origin	Human
Additional origin info	Female, Caucasian
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Nucleofection with non-integrating episomal plasmids carrying *OCT3/4, SOX2, KLF4, L-MYC, LIN28* and *shP53*
Genetic modification	NA
Type of modification	NA
Associated disease	Autosomal recessive Bardet-Biedl syndrome
Gene/locus	*BBS1,* Chr11: g.66293652 T > G, p.(Met390Arg); g.66293618G > C, p.(Gly379Arg); compound heterozygous. Ref sequence: NM_024649.4
Method of modification	NA
Name of transgene or resistance	NA
Inducible/constitutive system	NA
Date archived/stock date	25-01-2018
Cell line repository/bank	NA
Ethical approval	The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (H-3-2014-140). Written informed consent was obtained from the patients.

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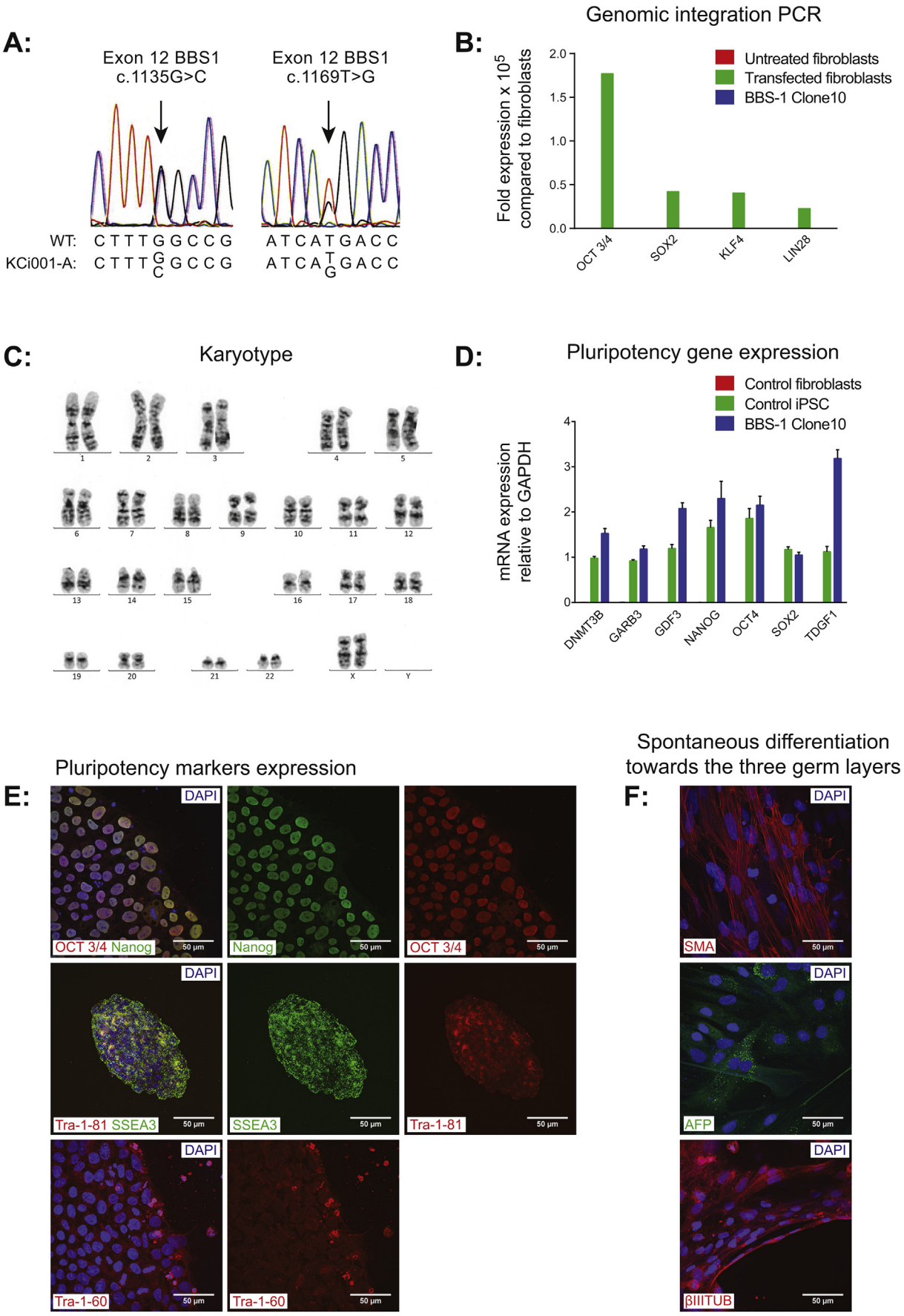

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Fig. 1. Characterization of BBS1 Clone 10 line (KCi001-A). A. WT sequences and mutations in KCi001-A in the same regions of the BBS1 gene. B. Quantitative real time PCR showing absence of episomal plasmids in BBS1 Clone 10 (KCi001-A) and control fibroblasts and presence of plasmids in transfected fibroblasts. C. Karyotype of representative metaphase showing normal 46 chromosomes (XX). D. mRNA expression of pluripotency markers in control iPSC line and in BBS1 Clone 10 (KCi001-A). E. Confocal images showing immunodetection of pluripotency-associated markers in BBS1 Clone 10 (KCi001-A). F. Immunofluorescence analysis of in vitro differentiation of BBS1 Clone 10 (KCi001-A) EBs using specific antibodies against the mesodermal marker α-smooth muscle actin (SMA), endodermal marker α-fetoprotein (AFP) and the ectodermal marker βIII-tubulin (βtub). Nuclei were stained with DAPI. Scale bar 50μM.

1. Resource utility

Bardet-Biedl syndrome BBS is characterized by defective cilia function. The *BBS1* encoded protein is a member of the BBSome complex, which is important for trafficking of membrane proteins in the cilium. The generated iPSC line represents a useful source to investigate the effect of *BBS1* in the pathophysiology of BBS.

2. Resource details

BBS is a rare, autosomal recessive disorder, where cellular, cilium-dependent signalling is affected. It has an estimated prevalence of 1/59000 in Denmark (Hjortshøj et al., 2010). Primary cilia are microtubule-based organelles, extending from the surface of most quiescent vertebrate cells. The BBS1 protein is part of a protein complex termed the BBSome and is thought to have a function in intra flagellar transport (IFT) in the primary cilium and in the connecting cilium, a specialized primary cilium in photoreceptors. Previous studies have shown defective IFT as a result of pathogenic variants in the genes encoding the proteins of the BBSome complex (Nager et al., 2017), but few studies have focused on the function of BBS1 even though it is one of the most frequently affected protein in BBS (Forsythe and Beales, 2012).

Fibroblasts were obtained from a skin biopsy of a patient with classic BBS symptoms, and compound heterozygous for the *BBS1* variants: c.1169T > G, p.(Met390Arg) and c.1135G > C, p. (Gly370Arg). The fibroblasts were reprogrammed into iPSC by nucleofection of three non-integrating episomal plasmids, encoding the human genes; *OCT3/4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28* and a p53 knock down *shP53*. Successful isolation of the iPSC clone, KCi001-A was achieved and sequencing of genomic DNA from KCi001-A confirmed the retention of the *BBS1* variants (Fig. 1A). Absence of integration of the episomal plasmids in genomic DNA in KCi001-A, were verified by quantitative RT-PCR analysis (Applied Biosystems 7500 Fast system) using plasmid specific primers for *OCT3/4*, *SOX-2*, *KLF4*, *LIN28* and *L-MYC* (Table 2), in the presence of SYBR green (Fig. 1B). DNA from control fibroblast 72 h post transfection and DNA from un-transfected fibroblasts were used as a positive and negative control, respectively (Fig. 1B). Primers for the corresponding endogenous genes were included as positive controls (not shown). Normal karyotype of the generated iPSC was preserved (46,XX) (Fig. 1C). Short tandem repeat (STR)-PCR profile analysis, where 22 different loci were analysed, showed 100% identity match between the parental fibroblast cell line and KCi001-A (submitted in archive with journal). Pluripotency was confirmed by expression of the genes, *OCT4*, *NANOG*, *SOX-2*, *TDGF1*, *DNMT3B*, *GDF3*and *GARB3*, analysed by RT-qPCR using Taq-man probes (Fig. 1D). The mRNA level of the different genes, were normalized to *GAPDH* mRNA. RNA from untreated fibroblasts and from a control iPSC line was included as a negative and a positive control, respectively (Fig. 1D). Pluripotency was further supported by immunocytochemistry (ICC) demonstrating the presence of the proteins Nanog and Oct4 in the nucleus, and the surface epitopes recognized by SSEA3, Tra-1-60 and Tra-1-81 antibodies in the cytoplasm (Fig. 1E). Furthermore, the capability of KCi001-A to differentiate into cells of all three germ layers was carried out by spontaneous differentiation, initiated by embryoid body formation, followed by adherent culture for a total of 21 days. The iPSC line showed positive ICC staining for mesodermal (α-smooth muscle actin (SMA)), endodermal (α-fetoprotein (AFP)) and ectodermal markers (βIII-tubulin (βtub)) (Fig. 1F). All together these data show that we have successfully created a BBS1 iPSC line; KCi001-A (Table 1).

3. Materials and methods

3.1. Reprogramming

Fibroblasts, were grown in DMEM-F12 + GlutaMAX (Gibco), 10% foetal bovine serum (FBS), 1% penicillin-streptomycin (Gibco) in a 37 °C humidified 5%-CO_2 incubator. 5×10^5 cells were transfected with 1,25 ng of each of the three plasmids; hOct3/4, hSK, hUL (Addgene plasmids #27077, #27078, #27080) in Primary Mammalian Fibroblasts buffer (Lonza), using Amaxa Nucleofector™ 2b/program V-

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal ES-like morphology	Not shown
Phenotype	Immunocytochemistry	Positive for cell surface markers; Oct4, Nanog, SSEA3, Tra-1-60, Tra-1-81	Fig. 1 panel E
	RT-qPCR (TaqMan probes; Applied Biosystems 7500 Fast system)	Positive for; OCT4, NANOG, SOX2, TDGF1, DNMT3B, GARB3 and GDF3	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46,XX, resolution 450–500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	NA	NA
	STR analysis Elucigene QST*R PLUSv2	22 sites were tested. 100% identity match between parental fibroblasts and KCi001-A	submitted in archive with journal
Variant analysis	Sanger sequencing	GENE: *BBS1* Compound heterozygous Chr11: g.66293652 T > G, p.(Met390Arg)/g.66293618G > C, p.(Gly379Arg)	Fig. 1 panel A
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR (negative)	Supplementary File 1
Differentiation potential	Embryoid body formation	Presence of the proteins α-smooth muscle actin (SMA), α-fetoprotein (AFP) and βIII-tubulin (βtub) were used to confirm formation of the three germ layers.	Fig. 1 panel F
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

024 and seeded on gelatine coated dishes (Sigma) in fibroblast medium without antibiotics for 24 h followed by culturing in standard fibroblast medium. On day 6 after transfection, iPSCs were seeded on ESC grade Matrigel (Corning) coated dishes, 50–80 × 10^3 cells/well, in mTeSR1 media (stem cell Technology) in an atmosphere of 5% CO_2, 5% O_2, 90% N_2 at 37 °C. From day 20 iPSC colonies were manually dissected. The iPSCs were split using 0,5 mM ultrapure EDTA (Gibco) at 70% confluency and frozen in mTeSR1 with 10% DMSO.

3.2. Karyotyping

Cells were treated with KaryoMAX colcemid for 45 min, dissociated and treated with hypotonic solution followed by fixation in freshly mixed 75% methanol: 25% acetic acid. Metaphase chromosomes were stained with Giemsa for cytogenic analysis.

3.3. Genomic DNA isolation, integration, genotyping and short tandem repeat (STR) analyses

DNA was purified using DNeasy Blood and Tissue kit (QIAGEN). Integration analysis was performed by Quantitative PCR using plasmid specific primers in the presence of SYBR green (Table 2). Data were analysed using the ΔΔCT method and the amounts were normalized to GAPDH. To confirm the identity of the cell-line, genotyping was performed with BBS1 specific primers and STR was performed using Elucigene QST*R PLUSv2.

3.4. Quantitative real time reverse transcriptase polymerase chain reaction (RT-qPCR)

RNA was harvested using RNeasy kit (QIAGEN), treated with DNase I (Invitrogen,) and cDNA synthesized using high capacity cDNA kit (Applied Biosystems). Taqman probes (Table 2) were used. Relative standard curves were used for data analysed and amounts normalized to GAPDH mRNA.

3.5. In vitro differentiation

The iPSC were treated with 0,5 mM ultrapure EDTA (Gibco) and plated in ultra-low adhesion plates (CORNING) in mTeSR1 media with ROCK inhibitor, to induce formation of embryoid bodies. On day two media was changed to DMEM-F12 + GlutaMAX (Gibco), 20% knock-out serum replacement (Gibco), 1 × non-essential amino acids (Sigma), 0,1 mM 2-mecaptoethanol (Sigma) and 1% pen/strep After one week of suspension culture the aggregates were transferred to adherent culture in DMEM F-12 with GlutaMAX, 10% FBS and 1% pen/strep media on

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Rabbit anti-NANOG	1:500	PeproTech Cat# 500-P236, RRID: AB_1268805
Pluripotency marker	Mouse anti-OCT3/4	1:200	Santa Cruz Biotechnology Cat# sc5279, RRID: AB_628051
Pluripotency marker	Rat anti SSEA3	1:200	BioLegend Cat# 330302, RRID: AB_1236554
Pluripotency marker	Mouse anti-TRA-1-60	1:200	BioLegend Cat# 330602, RRID: AB_1186144
Pluripotency marker	Mouse anti-TRA-1-81	1:200	BioLegend Cat# 330702, RRID: AB_1089240
Differentiation marker, Mesoderm	Mouse anti- α-smooth muscle actin (SMA)	1:500	Dako Cat# M0851, RRID: AB_2223500
Differentiation marker, Endoderm	Rabbit anti-α-fetoprotein (AFP)	1:500	Dako Cat# A0008, RRID: AB_2650473
Differentiation marker, Ectoderm	Mouse anti-βIII tubulin (βtub)	1:4000	Sigma-Aldrich Cat# T8660, RRID: AB_477590
Secondary antibody	Alexa Flour Goat Anti-Rabbit 488	1:800	Life Technologies Cat# A11008, RRID: AB_143165
Secondary antibody	Alexa Flour Donkey Anti-Mouse 546	1:800	Life Technologies Cat# A10036, RRID: AB_2534012
Secondary antibody	Alexa Flour Rabbit Anti-Rat 488	1:800	Molecular Probes Cat# A-21210, RRID: AB_2535796

Primers

	Target	Forward/Reverse primer (5′-3′)
Episomal Plasmids (qPCR)	OCT3/4 Plasmid	CATTCAAACTGAGGTAAGGG/TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	OCT3/4 Endogenous	CCCCAGGGCCCCATTTTGGTACC/ACCTCAGTTTGAATGCATGGGAGAGC
Episomal Plasmids (qPCR)	KLF4 Plasmid	CCACCTCGCCTTACACATGAAGA/TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	KLF4 Endogenous	ACCCATCCTTCCTGCCCGATCAGA/TTGGTAATGGAGCGGCGGGACTTG
Episomal Plasmids (qPCR)	SOX2 Plasmid	TTCACATGTCCCAGCACTACCAGA/TTTGTTTGACAGGAGCGACAAT
Endogenous (qPCR)	SOX2 Endogenous	TTCACATGTCCCAGCACTACCAGA/TCACATGTGTGAGAGGGGCAGTGTGC
Episomal Plasmids (qPCR)	L-MYC Plasmid	GGCTGAGAAGAGGATGGCTAC/TTTGTTTGACAGGAGCGACAAT
Endogenous (qPCR)	L-MYC Endogenous	GCGAACCCAAGACCCAGGCCTGCTCC/CAGGGGGTCTGCTCGCACCGTGATG
Episomal Plasmids (qPCR)	LIN28 Plasmid	AGCCATATGGTAGCCTCATGTCCGC/TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	LIN28 Endogenous	AGCCATATGGTAGCCTCATGTCCGC/TCAATTCTGTGCCTCCGGGAGCAGGGTAGG
House-Keeping Gene (qPCR)	GAPDH (1)	ACCACAGTCCATGCCATCAC/TCCACCACCCTGTTGCTGTA
BBS1 patogenic variants (Seq.)	BBS1 ex 12	GTGAGATTGGAGGGGAGATG/GGGATGCTGGGTGAACTAGA

Taqman probes

	Target	Assay ID
Pluripotency marker (RT-qPCR)	POU5F1/OCT4	Thermo Fisher Scientific Hs04260367_g1
Pluripotency marker (RT-qPCR)	NANOG	Thermo Fisher Scientific Hs04260366_g1
Pluripotency marker (RT-qPCR)	SOX2	Thermo Fisher Scientific Hs01053049_s1
Pluripotency marker (RT-qPCR)	TDGF1	Thermo Fisher Scientific Hs02339497_g1
Pluripotency marker (RT-qPCR)	DNMT3B	Thermo Fisher Scientific Hs00171876_m1
Pluripotency marker (RT-qPCR)	GARB3	Thermo Fisher Scientific Hs00241459_m1
Pluripotency marker (RT-qPCR)	GDF3	Thermo Fisher Scientific Hs00220998_m1
House-Keeping Gene (RT-qPCR)	GAPDH	Thermo Fisher Scientific Hs99999905_m1

gelatine (Sigma) coated coverslips. Morphological changes were observed and after two weeks of adherent culture the cells were fixed and investigated by immunocytochemistry.

3.6. Immunocytochemistry

Cells grown on gelatine or matrigel (for pluripotency) coated coverslips were fixed with 4% paraformaldehyde (Hounisen) for 15 min, and permeabilized with 0,2% TritonX-100 in PBS for 15 min. Slides were incubated in blocking buffer for 1 h (3% BSA, 0,2% TritonX-100 in PBS). Incubation with primary antibodies diluted in blocking buffer, was performed for 2 h at RT or overnight at 5 °C followed by incubation with secondary antibodies in blocking buffer (Table 2). Nuclei were stained using DAPI.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.08.005.

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